

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Ultra-low dose (+)-naloxone restores the thermal threshold of morphine tolerant rats[☆]



Kuang-Yi Chou^a, Ru-Yin Tsai^{a,b,c,g}, Wei-Yuan Tsai^b,
Ching-Tang Wu^d, Chun-Chang Yeh^d, Chen-Hwan Cherng^{d,**},
Chih-Shung Wong^{b,c,e,f,*}

^a General Education Center, National Taipei University of Nursing and Health Sciences, Taipei, Taiwan

^b Department of Anesthesiology, Cathay General Hospital, Taipei, Taiwan

^c Neuropathic Pain and Translational Medicine Research Laboratory, Cathay General Hospital, Taipei, Taiwan

^d Department of Anesthesiology, Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan

^e School of Medicine, Fu-Jen Catholic University, New Taipei, Taiwan

^f Graduate Institute of Medical Science, National Defense Medical Center, Taipei, Taiwan

Received 26 August 2013; received in revised form 19 November 2013; accepted 20 November 2013

KEYWORDS

morphine-tolerance;
intrathecal injection;
neuropathy;
antinociception

Background/purpose: As known, long-term morphine infusion leads to tolerance. We previously demonstrated that both co-infusion and post-administration of ultra-low dose (\pm)-naloxone restores the antinociceptive effect of morphine in morphine-tolerant rats. However, whether the mechanism of the action of ultra-low dose (\pm)-naloxone is through opioid receptors or not. Therefore, in the present study, we further investigated the effect of ultra-low dose (+)-naloxone, it does not bind to opioid receptors, on the antinociceptive effect of morphine.

Methods: Male Wistar rats were implanted with one or two intrathecal (i.t.) catheters; one catheter was connected to a mini-osmotic pump, used for morphine (15 μ g/h), ultra-low dose (+)-naloxone (15 pg/h), morphine plus ultra-low dose (+)-naloxone (15 pg/h) or saline (1 μ l/h) infusion for 5 days. On day 5, either ultra-low dose (+)-naloxone (15 pg) or saline (5 μ l) was injected via the other catheter immediately after discontinued morphine or saline infusion.

[☆] Conflict of interest: all authors have declared no conflicts of interest exist.

* Corresponding author. Department of Anesthesiology, Cathay General Hospital, 280, Renai Road, Section 4, Taipei, Taiwan. Tel.: +886 2 27082121x3510; fax: +886 2 87924835.

** Corresponding author.

E-mail addresses: cherng1018@yahoo.com.tw (C.-H. Cherng), w82556@gmail.com (C.-S. Wong).

[‡] Contributed equally to this work as first authors.

Three hours later, morphine (15 μ g in 5 μ l saline) or saline were given intrathecally. All rats received nociceptive tail-flick test every 30 minutes for 120 minutes after morphine challenge at different temperature (45–52°C, respective).

Results: Our results showed that, both co-infusion and post-treatment of ultra-low dose (+)-naloxone with morphine preserves the antinociceptive effect of morphine. Moreover, in the post administration rats, ultra-low dose (+)-naloxone further enhances the antinociceptive effect of morphine.

Conclusion: This study provides an evidence for ultra-low dose (+)-naloxone as a therapeutic adjuvant for patients who need long-term opioid administration for pain management.

Copyright © 2013, Elsevier Taiwan LLC & Formosan Medical Association. All rights reserved.

Introduction

Morphine is the most widely used analgesic for treating moderate to severe pain in clinical practice. However, long-term morphine administration induces tolerance, which hampers its clinical use. The mechanisms postulated to explain morphine tolerance include G protein uncoupling,¹ opioid receptor internalization,² down-regulation of opioid receptors^{3,4} and glutamate transporters,⁵ and upregulation of N-methyl-D-aspartate (NMDA) receptors.^{6,7} It has been suggested that spinal cord glia astrocytes and microglia contribute to the development of morphine tolerance and tolerance associated pain sensitization. Co-administration of a second drug with an opioid is a strategy for enhancing the antinociceptive effect of morphine, which also can attenuate tolerance.^{4,8}

Ultra-low dose (\pm)-naloxone, had been demonstrated as an effective adjuvant with opioids to suppress opioid tolerance and dependence, re-initiation of the classical μ -opioid receptor- G_i protein coupling signaling was observed in chronic morphine-treated rats.⁹ Ultra-low dose (\pm)-naloxone prevents the μ -opioid receptor coupling switches from G_i to G_s protein, thus attenuating tolerance development, possibly via naloxone binds to the C-terminal of a scaffolding protein filamin A which interacts with the μ -opioid receptors.¹⁰ Furthermore, intrathecal co-administration of naloxone (20 ng) with morphine (2 mg) significantly improved the pain relief in patients with severe chronic low back pain.¹¹ In agreement with these reports, we previously also demonstrated that intrathecal co-infusion of ultra-low dose (\pm)-naloxone with morphine, significantly inhibited the development of tolerance in accompanied with reduction of CSF excitatory amino acid glutamate and aspartate concentration, suppression of microglia activation, and down-regulation of proinflammatory cytokine levels in the spinal cord microglia of chronic morphine-infused rats.^{12,13}

On the basis of the evidence for glia cell activation during the development of morphine tolerance and the modulating effect of ultra-low dose naloxone on the antinociceptive effect of morphine, we suggest that co-treatment of ultra-low dose naloxone with morphine might maintain the normal microenvironment of neuron and glia cells, thus restores the antinociceptive effect of morphine. In the present study, we investigated the effect of ultra-low dose (+)-naloxone, it does not act through opioid receptors,^{14,15} on restoration of the antinociception of morphine in morphine-tolerant rats.

Methods and materials

Animal preparation and intrathecal drug delivery

The use of rats in this study conformed to the Guiding Principles in the Care and Use of Animals of the American Physiology Society and was approved by the National Defense Medical Center Animal Care and Use Committee. Male Wistar rats (350–400 g; Biolasco Taiwan Co., Taipei, Taiwan) were anaesthetized with phenobarbital (65 mg/kg, intraperitoneally) and implanted with one or two intrathecal catheters. The catheter was inserted via the atlantooccipital membrane down to the spinal cord segments L5, L6 and S1–S3 that relative to the tail flick reflex.¹⁶ One intrathecal catheter was connected to a mini-osmotic pump (Alzet, Cupertino, CA) for infusion of saline (1 μ l/h), or morphine (15 μ g/h; Sigma, Missouri, USA), or ultra-low dose (+)-naloxone (15 pg/h; generous gift from Dr. Jau-Shyong Hong, National Institutes of Health, Research Triangle Park, North Carolina, USA), or morphine plus ultra-low dose (+)-naloxone (15 pg/h) at the rate of 1 μ l/h for 5 days. After catheterization (day 0), all rats were returned to their home cages for recovery. Each rat was housed individually and maintained on a 12 h light/dark cycle with food and water freely available. Rats with neurological deficits were excluded. On day 5 after morphine tolerance developed, (i) the morphine pluses ultra-low dose (+)-naloxone co-infusion rats were underwent a nociceptive tail-flick test. (ii) In the post-treatment rats; the intrathecal catheter for saline or morphine infusion was cut, and the rats were intrathecally injected with saline (5 μ l) or ultra-low dose (+)-naloxone (15 pg), and 30 min later, a single dose of morphine (15 μ g in 5 μ l saline, intrathecally) was injected and the antinociceptive effect was measured. All drugs were delivered intrathecally in 5 μ l and flushed by 8 μ l of saline. No abnormal motor function was observed in all rats after intrathecal test drug injection (data not shown).

Antinociceptive tests

Tail-flick latency, using the hot water immersion test (52 \pm 0.25°C), was measured before drug infusion and daily after start of infusion for 5 days. The temperature of water was controlled by Thermostatic Circular Water Bath with thermometer. The averaged baseline tail-flick latencies of all rats in the 52°C warm-water were 2 \pm 0.25 s. A latency of 10 seconds was set as the cut-off time to avoid tail damage.

The morphine challenge test (15 µg in 5 µl saline, intrathecally) was performed after the 5-day infusion; rats were placed in plastic restrainers for drug injection and antinociception measurement. The peak antinociceptive response to morphine challenge was observed at 30 min after injection in our previous studies.^{12,17} Different temperatures were used, 45–52°C ($n = 5$ /each temperature), to examine the thermal nociceptive threshold shift in morphine-tolerant rats and the effect of ultra-low dose (+)-naloxone treatment on the nociceptive effect of morphine in rats. The percentage of the maximal possible antinociceptive effect (% MPE) was calculated as (maximum latency – baseline latency)/(cut-off latency – baseline latency) × 100.

Construction of intrathecal catheter

The intrathecal catheter construction was followed as our previous study.^{18–20} The intrathecal catheter was constructed using an 8-cm polyethylene tube (0.008 inch inner diameter, 0.014 inch outer diameter; Spectranetics, Colorado springs, CO, USA) and a 3.5 cm silastic tube (Dow Corning, Midland, MI, USA). The silastic tube was inserted into the polyethylene tube and the joint sealed with epoxy resin and silicon rubber. The dead space of the intrathecal catheter was about 8 µl.

Statistical analysis

All data are presented as mean ± S.E.M. The statistical analysis was performed using SigmaStat 3.0 software (SYSTAT Software Inc., San Jose, CA). Behavior tests were analyzed using two-way (time and treatment) ANOVA with a *post hoc* Bonferroni correction followed by subsequent one-way ANOVA (at each time of the experiment). A significant difference was defined as a p -value < 0.05.

Results

Ultra-low dose (+)-naloxone co-infusion preserves the thermal antinociceptive effect of morphine

Rats receiving intrathecal morphine (15 µg/h) infusion for 5 days developed morphine tolerance as our previous

studies (data not shown).^{12,13,19,21–23} On day 5, different temperatures were used (45–52°C) to examine the thermal nociceptive threshold shift in morphine-tolerant rats and the effect of ultra-low dose (+)-naloxone treatment on the nociceptive effect of morphine in rats (Table 1). As shown in Fig. 1 chronic morphine infusion (Mo) showed a significant decreased nociceptive temperature threshold to 50% MPE of tail-flick latency from 50.0°C to 48.56°C ($p < 0.01$, compare with saline control group). While, co-infusion of ultra-low dose (+)-naloxone with morphine preserving the 50% MPE of tail-flick latency at 51.32°C ($p < 0.01$, compare with saline control and morphine groups). It suggests that co-infusion of ultra-low dose (+)-naloxone with morphine prevents the morphine tolerance development and maintains the antinociception of morphine.

Ultra-low dose (+)-naloxone restores the antinociceptive effect of morphine in morphine-tolerant rats

On day 5, nociceptive tolerance was developed after continued morphine (15 µg/h) infusion. At 3 hours after discontinuation of morphine infusion, intrathecal ultra-low dose (+)-naloxone (15 pg) was pretreated 30 minutes before morphine (15 µg) challenge, then the tail-flick latency (45–52°C) was performed (Table 2), the nociceptive baseline was 1.98 ± 0.37 seconds. Similar to our previous study,⁷ morphine challenge on day 5 (Fig. 2) produced a significant antinociceptive effect in saline-infused rats (9.95 ± 0.2 sec; Sal/Mo), but not in morphine-tolerant rats (0.9 ± 0.31 sec; Mo/Mo). As expected, ultra-low dose (+)-naloxone (15 pg) alone had no antinociceptive effect in either saline-infused controls (data not shown; Sal/NLX) or morphine-tolerant rats (2.24 ± 0.24 sec; Mo/NLX). In contrast, ultra-low dose (+)-naloxone alone restored the nociceptive threshold to the control level ($p = 0.875$, compared with saline control group). Furthermore, pretreatment of ultra-low dose (+)-naloxone (15 pg), before morphine challenge, significantly restored the antinociceptive effect of morphine (5.02 ± 0.74 sec; Mo/NLX/Mo). Identically, pretreatment of ultra-low dose (+)-naloxone (15 pg) also significantly restores the 50% MPE of tail-flick latency from 48.30°C (Mo/Mo) back to 52.02°C ($p < 0.01$, compare with morphine tolerance group). It suggests that pretreatment with ultra-low

Table 1 Tail-flick latency (sec) of temperature response in different-treated rats.

	Sal	NLX	Mo	Mo/NLX
45°C	9.98 ± 0.02	9.96 ± 0.09	9.83 ± 0.41	10 ± 0
46°C	9.8 ± 0.63	9.75 ± 0.53	9.15 ± 1.01	9.95 ± 0.17
47°C	9.78 ± 0.62	9.68 ± 0.79	7.33 ± 0.8**	9.88 ± 0.37##
48°C	7.72 ± 0.5	7.52 ± 1.2	5.55 ± 0.57**	9.29 ± 1.39##
49°C	7.31 ± 0.37	7.11 ± 1.44	4.91 ± 0.9**	8.86 ± 1.22##
50°C	5.0 ± 0.32	5.21 ± 1.12	3.42 ± 0.98*	6.33 ± 1.26##
51°C	4.23 ± 0.93	4.03 ± 0.9	1.963 ± 0.56**	5.8 ± 1.1##
52°C	2.05 ± 0.07	2.25 ± 0.17	1.24 ± 0.24**	4.42 ± 0.94##

Tail-flick latency was performed on day 5 after drug infusion. All data points are the mean ± SEM for 5 rats per group. * $p < 0.05$;

** $p < 0.01$ compared to the saline-infusion group.

$p < 0.01$ compared to the morphine-infusion group.

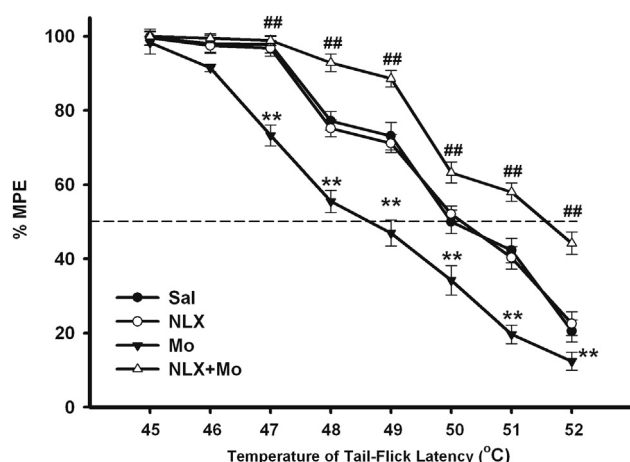


Figure 1 Ultra-low dose (+)-naloxone attenuated morphine tolerance. Temperature-response curves for the antinociceptive effect of morphine constructed from the tail-flick test results were calculated from data on day 5 of drug infusion. All data points are the mean \pm SEM and averaged from the indicated number of rats. ** $p < 0.01$ compared to the saline-infusion group. ## $p < 0.01$ compared to the morphine-infusion group. The dashed line indicates 50% MPE. Sal = saline; NLX = ultra-low dose (+)-naloxone; Mo = morphine; Mo + NLX = morphine + ultra-low dose (+)-naloxone.

dose (+)-naloxone capable restores the nociceptive threshold to the control baseline and further restores the antinociception of morphine in morphine-tolerant rats.

Discussions

This study shows that ultra-low dose (+)-naloxone preserves the antinociceptive effect of morphine both in co-treatment and post-treatment with morphine in morphine-tolerant rats. Moreover, co-treatment ultra-low dose (+)-naloxone with morphine further prevents morphine tolerance development in rats.

Morphine is the most effective drug for pain relief. However, repeated use of morphine induces tolerance to its analgesic effect. The underlying mechanisms of morphine tolerance are complex, co-administration of a second drug

with morphine is a strategy for enhancing the antinociceptive effect and attenuating the development of tolerance.^{7,21,24} Our previous studies indicated that dexamethasone co-infusion with morphine reduces morphine-evoked excitatory amino acid release and downregulates glutamate transporters GLT-1 and GLAST expression²⁵ and co-infusion of amitriptyline with morphine maintains the antinociceptive efficacy of morphine by attenuation of morphine-induced neuroinflammation^{19,22} and NMDA receptor NR1 subunit and PKC γ expression.²¹ Ultra-low dose (\pm)-naloxone, use as an effective adjuvant with opioids to suppress opioid dependence and tolerance, it has been shown to be associated with switches in μ -opioid receptor-G coupling and G β/γ subunit signaling in chronic morphine-tolerant rats.⁹ Ultra-low dose (\pm)-naloxone prevents the coupling switch of G i/s protein, thus attenuating opioid tolerance, via regulating interaction of naloxone with C-terminal of filamin A.¹⁰ Moreover, our recently studies also provide evidence that ultra-low dose (\pm)-naloxone preserves the antinociceptive effect of morphine via attenuation of NMDA receptor neurotransmission and suppression of neuroinflammation in morphine-tolerant rats.^{12,13} Furthermore, intrathecal naloxone (20 ng) with morphine (2 mg) has been reported to produce a dramatic improvement in pain management in humans with severe chronic low back pain.¹¹ These results indicate that the combination of ultra-low dose (\pm)-naloxone with morphine can preserve the analgesic effect of morphine, possibly through modulation of multiple signal transduction pathways.

Although the success of ultra-low dose (\pm)-naloxone in preventing morphine tolerance development is consistently reported for studies in which ultra-low dose (\pm)-naloxone was administered by continuous infusion,^{12,13} however, we did not know whether the preventing of morphine tolerance and anti-neuroinflammatory effect of ultra-low dose (\pm)-naloxone was involved in the opioid receptor-mediated signaling pathway. The present study proved the effect was due to mechanisms not related to the opioid receptors because (+)-naloxone does not bind to the opioid receptors.^{14,15} Studies had found that both (–)-naloxone and (+)-naloxone significantly attenuated β -amyloid peptide-induced superoxide production in microglia cells and that the anti-inflammatory effect of naloxone may not be directly related to opioid receptors,²⁶ as (+)-naloxone, which does not bind to opioid receptors, also had a

Table 2 Tail-flick latency (sec) of temperature response after morphine challenge in rats.

	Sal/Sal	Sal/Mo	Mo/Mo	Mo/NLX	Mo/NLX/Mo
45°C	9.99 \pm 0.02	10 \pm 0	9.03 \pm 0.61	10.0 \pm 0	10.0 \pm 0
46°C	9.78 \pm 0.53	10 \pm 0	8.05 \pm 0.35**	9.75 \pm 0.12	10 \pm 0##
47°C	9.67 \pm 0.60	9.99 \pm 0.01	7.03 \pm 0.5**	8.58 \pm 0.33##	9.78 \pm 0.23##
48°C	7.83 \pm 0.24	9.98 \pm 0.01**	5.50 \pm 0.53**	7.22 \pm 0.19##	8.99 \pm 0.79##
49°C	6.51 \pm 0.31	9.98 \pm 0.02**	4.01 \pm 1.2**	5.56 \pm 0.7##	8.06 \pm 0.22##
50°C	4.80 \pm 0.12	9.98 \pm 0.02**	3.42 \pm 0.28**	4.63 \pm 0.56##	7.03 \pm 0.66##
51°C	3.75 \pm 0.95	9.97 \pm 0.02**	1.95 \pm 0.26**	3.54 \pm 0.89##	6.18 \pm 0.68##
52°C	1.98 \pm 0.37	9.95 \pm 0.2**	0.9 \pm 0.31**	2.22 \pm 0.24##	5.02 \pm 0.74##

Tail-flick latency was performed on day 5 after morphine challenge. All data points are the mean \pm SEM for 5 rats per group.

** $p < 0.01$ compared to the saline-infusion group.

$p < 0.01$ compared to the morphine-infusion group.

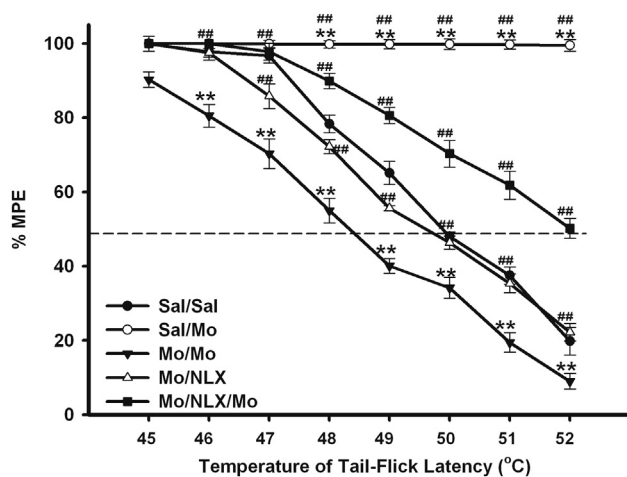


Figure 2 Ultra-low dose (+)-naloxone restored the antinociceptive effect of morphine. Morphine's antinociceptive effect was examined on day 5 after intrathecal either saline (1 μ l/h) or morphine (15 μ g/h) infusion. At 3 hours after discontinuation of infusion, the rats were intrathecally injected either saline or ultra-low dose (+)-naloxone (15 μ g) 30 minutes before morphine challenge (15 μ g), by which the tail-flick latencies had returned to less than 3 seconds, then the tail-flick latency was measured every 30 minutes for 120 minutes. Temperature-response curves for the antinociceptive effect of morphine constructed from the tail-flick test results were calculated from data at 60 minutes after morphine challenge. All data points are the mean \pm SEM of five rats per group. ** p < 0.01 compared to the saline-infusion group. ## p < 0.01 compared to the morphine-infusion group. The dashed line indicates 50% MPE. Sal/Sal = saline-infusion plus saline challenge; Sal/Mo = saline-infusion plus morphine challenge; Mo/Mo = morphine-infusion plus morphine challenge; Mo/NLX = morphine-infusion plus ultra-low dose (+)-naloxone pretreatment; Mo/NLX/Mo = morphine-infusion plus morphine challenge with ultra-low dose (+)-naloxone pretreatment.

remarkable anti-inflammatory effect. Moreover, (+)-naloxone was found to reduce cocaine-induced and amphetamine-induced hyperactivity in mice^{27,28}. Our present study demonstrated that 15 μ g (+)-naloxone not only prevented morphine tolerance development but also enhanced the antinociceptive effect of morphine in morphine-tolerant rats. Together, these results raise the possibility that ultra-low dose (+)-naloxone acts by binding to sites other than opioid receptors.

In our previous studies, we found that ultra-low dose (\pm)-naloxone cotreatment with morphine can inhibit neuropathic thermal hyperalgesia,^{20,29} which might be through its anti-inflammatory action on the spinal cord microglia and reinitiate the classical μ -opioid receptor and G_i -protein coupling in the pertussis toxin-induced neuropathic pain rats. These results suggest that ultra-low dose (\pm)-naloxone restores the opioid signaling in pertussis toxin-treated rats to that of normal rats, thus reversing the antinociceptive effect of morphine via the inhibitory μ -opioid receptor/ G_i -protein signal transduction pathway. In our present study, ultra-low dose (+)-naloxone cotreatment with morphine significant

prevented morphine tolerance development and further enhanced the antinociceptive effect of morphine. Moreover, in morphine-tolerant rats, ultra-low dose (+)-naloxone completely recovered the tail-flick latency to that of control baseline and further enhanced the antinociceptive effect of morphine in morphine tolerant rats. Our results indicate that ultra-low dose (+)-naloxone might regulate μ -opioid receptor/ G_i -protein coupling via different mechanisms other than the opioid-mediated actions. This study provides new evidence that ultra-low dose (+)-naloxone may have potential as an analgesic adjuvant in clinical pain management, particularly for patients who need long-term morphine treatment and morphine-tolerant patients who require better pain relief.

Acknowledgments

This study was supported by grants from the National Science Council (NSC-101-2314-B-281-001-MY3), Cathay General Hospital, Taipei, Taiwan (CGH-MR-A10206) and was performed at the Neuropathic Pain and Translational Medicine Research Laboratory, Cathay Medical Research Institute, Cathay General Hospital, Xizhi, New Taipei City, Taiwan.

References

- Chakrabarti S, Regec A, Gintzler AR. Biochemical demonstration of mu-opioid receptor association with G α 12/13: enhancement following morphine exposure. *Brain Res Mol Brain Res* 2005;135:217–24.
- Zuo Z. The role of opioid receptor internalization and beta-arrestins in the development of opioid tolerance. *Anesth Analg* 2005;101:728–34.
- Wong CS. The mechanisms of ultra-low dose opioid agonist-antagonist. *J Formos Med Assoc* 2011;110: 666; author reply 667.
- Yeh YC, Lin TF, Chang HC, Chan WS, Wang YP, Lin CJ, et al. Combination of low-dose nalbuphine and morphine in patient-controlled analgesia decreases incidence of opioid-related side effects. *J Formos Med Assoc* 2009;108:548–53.
- Tai YH, Wang YH, Wang JJ, Tao PL, Tung CS, Wong CS. Amitriptyline suppresses neuroinflammation and up-regulates glutamate transporters in morphine-tolerant rats. *Pain* 2006;124:77–86.
- Tai YH, Cheng PY, Tsai RY, Chen YF, Wong CS. Purinergic P2X receptor regulates N-methyl-D-aspartate receptor expression and synaptic excitatory amino acid concentration in morphine-tolerant rats. *Anesthesiology* 2011;113:1163–75.
- Tsai RY, Chou KY, Shen CH, Chien CC, Tsai WY, Huang YN, et al. Resveratrol regulates N-methyl-d-aspartate receptor expression and suppresses neuroinflammation in morphine-tolerant rats. *Anesth Analg* 2012;115:944–52.
- Yeh YC, Lin TF, Wang CH, Wang YP, Lin CJ, Sun WZ. Effect of combining ultralow-dose naloxone with morphine in intravenous patient-controlled analgesia: the cut-off ratio of naloxone to morphine for antiemesis after gynecologic surgery. *J Formos Med Assoc* 2008;107:478–84.
- Wang HY, Friedman E, Olmstead MC, Burns LH. Ultra-low-dose naloxone suppresses opioid tolerance, dependence and associated changes in mu opioid receptor-G protein coupling and Gbetagamma signaling. *Neuroscience* 2005;135:247–61.

10. Wang HY, Burns LH. Naloxone's pentapeptide binding site on filamin A blocks Mu opioid receptor-Gs coupling and CREB activation of acute morphine. *PLoS One* 2009;4:e4282.
11. Hamann S, Sloan PA, Witt W. Low-dose intrathecal naloxone to enhance intrathecal morphine analgesia: a case report. *J Opioid Manag* 2008;4:251–4.
12. Lin SL, Tsai RY, Shen CH, Lin FH, Wang JJ, Hsin ST, et al. Co-administration of ultra-low dose naloxone attenuates morphine tolerance in rats via attenuation of NMDA receptor neurotransmission and suppression of neuroinflammation in the spinal cords. *Pharmacol Biochem Behav* 2010;96:236–45.
13. Lin SL, Tsai RY, Tai YH, Cherng CH, Wu CT, Yeh CC, et al. Ultra-low dose naloxone upregulates interleukin-10 expression and suppresses neuroinflammation in morphine-tolerant rat spinal cords. *Behav Brain Res* 2010;207:30–6.
14. Lewis SS, Loram LC, Hutchinson MR, Li CM, Zhang Y, Maier SF, et al. (+)-naloxone, an opioid-inactive toll-like receptor 4 signaling inhibitor, reverses multiple models of chronic neuropathic pain in rats. *J Pain* 2012;13:498–506.
15. Hutchinson MR, Northcutt AL, Hiranita T, Wang X, Lewis SS, Thomas J, et al. Opioid activation of toll-like receptor 4 contributes to drug reinforcement. *J Neurosci* 2012;32:11187–200.
16. Grossman ML, Basbaum AI, Fields HL. Afferent and efferent connections of the rat tail flick reflex (a model used to analyze pain control mechanisms). *J Comp Neurol* 1982;206:9–16.
17. Tsai RY, Tai YH, Tzeng JI, Lin SL, Shen CH, Yang CP, et al. Ultra-low dose naloxone restores the antinociceptive effect of morphine in pertussis toxin-treated rats and prevents glutamate transporter downregulation by suppressing the p38 mitogen-activated protein kinase signaling pathway. *Neuroscience* 2009;159:1244–56.
18. Cherng CH, Lee KC, Chien CC, Chou KY, Cheng YC, Hsin ST, et al. Baicalin ameliorates neuropathic pain by suppressing HDAC1 expression in the spinal cord of spinal nerve ligation rats. *J Formos Med Assoc.* <http://dx.doi.org/10.1016/j.jfma.2013.04.007>; 2013.
19. Tai YH, Tsai RY, Wang YH, Cherng CH, Tao PL, Liu TM, et al. Amitriptyline induces nuclear transcription factor-kappaB-dependent glutamate transporter upregulation in chronic morphine-infused rats. *Neuroscience* 2008;153:823–31.
20. Tsai RY, Jang FL, Tai YH, Lin SL, Shen CH, Wong CS. Ultra-Low-Dose Naloxone Restores the Antinociceptive Effect of Morphine and Suppresses Spinal Neuroinflammation in PTX-Treated Rats. *Neuropsychopharmacology* 2008;33:2772–82.
21. Huang YN, Tsai RY, Lin SL, Chien CC, Cherng CH, Wu CT, et al. Amitriptyline attenuates astrocyte activation and morphine tolerance in rats: role of the PSD-95/NR1/nNOS/PKCgamma signaling pathway. *Behav Brain Res* 2012;229:401–11.
22. Tai YH, Tsai RY, Lin SL, Yeh CC, Wang JJ, Tao PL, Wong CS. Amitriptyline suppresses neuroinflammation-dependent interleukin-10-p38 mitogen-activated protein kinase-heme oxygenase-1 signaling pathway in chronic morphine infused rats. *Anesthesiology* 2009;110:1–11.
23. Tai YH, Wang YH, Tsai RY, Wang JJ, Tao PL, Liu TM, et al. Amitriptyline preserves morphine's antinociceptive effect by regulating the glutamate transporter GLAST and GLT-1 trafficking and excitatory amino acids concentration in morphine-tolerant rats. *Pain* 2007;129:343–54.
24. Chavooshi B, Saberi M, Pournaghash Tehrani S, Bakhtiarian A, Ahmadiani A, Haghparast A. Vigabatrin attenuates the development and expression of tolerance to morphine-induced antinociception in mice. *Pharmacol Biochem Behav* 2009;93:155–9.
25. Wen ZH, Wu GJ, Chang YC, Wang JJ, Wong CS. Dexamethasone modulates the development of morphine tolerance and expression of glutamate transporters in rats. *Neuroscience* 2005;133:807–17.
26. Liu Y, Qin L, Wilson BC, An L, Hong JS, Liu B. Inhibition by naloxone stereoisomers of beta-amyloid peptide (1-42)-induced superoxide production in microglia and degeneration of cortical and mesencephalic neurons. *J Pharmacol Exp Ther* 2002;302:1212–9.
27. Chatterjee N, Alexander GJ, Sechzer JA, Lieberman KW. Prevention of cocaine-induced hyperactivity by a naloxone isomer with no opiate antagonist activity. *Neurochem Res* 1996;21:691–3.
28. Chatterjee N, Sechzer JA, Lieberman KW, Alexander GJ. Dextro-naloxone counteracts amphetamine-induced hyperactivity. *Pharmacol Biochem Behav* 1998;59:271–4.
29. Tsai RY, Tai YH, Tzeng JI, Cherng CH, Yeh CC, Wong CS. Ultra-low dose naloxone restores the antinociceptive effect of morphine in pertussis toxin-treated rats by reversing the coupling of mu-opioid receptors from Gs-protein to coupling to Gi-protein. *Neuroscience* 2009;164:435–43.